Cell

A Common Mechanism that Underpins Antibody Diversification

Cornelis Murre[1,*]
[1]Department of Molecular Biology, Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093, USA
*Correspondence: cmurre@ucsd.edu
http://dx.doi.org/10.1016/j.cell.2015.10.075

Targeting of AID to antibody variable (V) regions results in somatic hypermutation, whereas its recruitment to switch (S) regions leads to class-switch recombination. Yeap et al. find that the mechanism by which variable and switch regions recruit AID essentially is the same but that the two regions differ in the density of double-stranded DNA breaks that are generated. These lead to either point mutations in V exons in somatic hypermutation or deletion of intervening DNA sequences during class switch recombination.

During the developmental progression of B-lineage cells, antibody genes are being assembled by unique combinations of gene segments encoding for variable (V), diversity (D), and joining (J) elements, a process frequently referred to as V(D)J recombination. In the primary lymphoid organs such as the fetal liver and the bone marrow, VDJ recombination ultimately leads to a diverse antibody repertoire expressed on the cell surface of the antigen-inexperienced mature B cell population. Upon expression of an innocuous B cell receptor (BCR), naive B cells exit the primary lymphoid organs and migrate to the spleen and lymph nodes. Once exposed to invading pathogens, B cells move to specialized microanatomical structures, named germinal centers. Here, B cells are subject to two distinct sets of genomic modifications: class switch recombination (CSR) and/or somatic hypermutation (SHM) (Chandra et al., 2015). Somatic point mutations in germinal center B cells that lead to increased affinity of the antigen receptor are selected for further maturation, while mutations that lead to a decline in antigen receptor affinity are being depleted within the population. Frequently, but not exclusively, CSR is also initiated in the germinal centers and can be induced in vitro by culturing naive B cells in the presence of the appropriate stimuli. During CSR, dsDNA breaks are generated at highly repetitive sequences, named S regions, that are located adjacent to exons encoding for the ensemble of antibody isotypes. DNA breaks generated during CSR find each other through thermal motion, leading to the joining of two S regions that replace the IgM constant region with that of other isotypes, including IgG, IgE, or IgA. Ultimately, this highly orchestrated series of genomic changes generates a population of effector B cells with distinct functions that express a diverse and high-affinity antibody repertoire.

The process of SHM and CSR requires activation-induced cytidine deaminase (AID). AID converts cytosine (C) into uracil (U), leading to DNA lesions (Muramatsu et al., 2007; Di Noia and Neuberger, 2007). Such DNA lesions generate double-stranded DNA breaks across switch regions or point mutations that are associated with exons encoding for V regions (Nussenzweig and Nussenzweig, 2010; Alt et al., 2013). Distinct DNA repair modules are used to repair and modify the DNA lesions, including mismatch DNA repair, base excision repair, and error-prone DNA polymerases.

AID predominantly, but not exclusively, targets RGYW consensus sequences (R relates to purine, Y to pyrimidine, W to A or T) (Hackney et al., 2009). The RGYW motif is enriched, albeit modestly, across the majority of the V region repertoire. The RGYW sequence is found in S regions more densely than within V exons. The high density of the RGYW consensus sequences at S regions and its palindromic nature permit the targeting of AID to both the sense and anti-sense DNA strands across the S regions (Zarrin et al., 2004). This then leads to double-stranded breaks and deletion of the intervening genomic sequences.

The RGWY motif (Figure 1) is not restricted to V exons. Rather it is present at other locations in the genome that are not subjected to AID targeting. If it is not specific to V exons and S regions, then what is the role of the RGYW motif in AID targeting? Furthermore, is the role of the RGWY motif equivalent to promote class switch recombination and somatic hypermutation? In this issue of *Cell*, Yeap et al. (2015) have designed an elegant experimental approach to address this question. Briefly, a strategy was developed to measure AID activity on a spectrum of test sequences, named passenger alleles. This assay permits the testing of a passenger against a V exon expressing a productive in-frame antibody allele in the same normal V-region genomic location and with the same transcriptional machinery (known to target AID). The mutations associated with passenger alleles were then compared to the sequences of a productive V region known to interact with a specific antigen, termed 4-hyroxy-3-nitrophenyacteyl (NP). This approach was combined with throughput DNA sequencing that permitted "surrogate kinetic" analyses of somatic mutations in both productive and passenger alleles. Furthermore, in the approach developed by Yeap et al. (2015), selection for NP does not occur within the time-frame that spans the short-term immunization protocols and that the passenger allele is not translated, and consequently, there is no bias by selection for antigen or against mutations that inactive the antibody. Interestingly, the authors found that AID

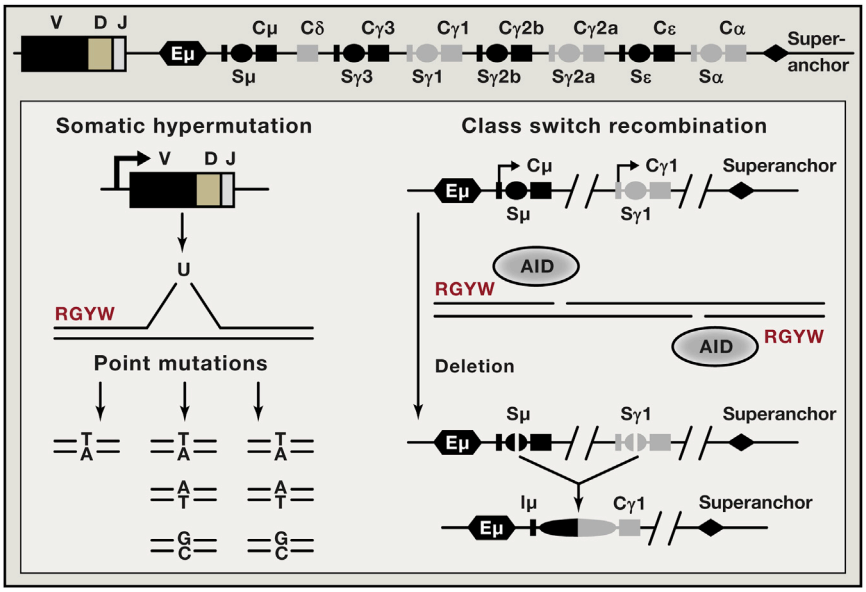

Figure 1. Schematic Diagram Depicting the Role of the RGYW Motif in Class-Switch Recombination and Somatic Hypermutation
Adapted and modified from Chandra et al. (2015).

acted on the most dominant RGYW targets in V exons and S regions, pointing to a conserved mechanism by which AID activates class switch recombination or somatic hypermutation. Given this striking similarity, the authors next addressed why targeting to V exons would yield point mutations rather than genomic deletions observed during class switch recombination. Using their reporter system, Yeap et al. (2015) observed that, surprisingly, somatic hypermutation did in fact lead to double-stranded breaks and genomic deletions in V exons, similar to class switch recombination process. Thus, no specialized mechanism is required for generating deletions that have been observed in subsets of antibodies, including those in neutralizing antibodies isolated from patients infected with HIV. These findings are intriguing, suggesting that the ability of S regions to be subjected to double-stranded breaks with limited AID exposure may have evolved to permit efficient class switch recombination, avoiding mutations associated with V regions that, in principle, could affect antibody affinity and/or specificity.

These data also provide insight into the special nature of V exons compared to other regions in the genome as it relates to being targeted by AID (Odegard and Schatz, 2006). The authors found that non-Ig DNA sequences, including bacterial DNA, that were inserted as passengers V-region location accumulated point mutations at RGWY sequences at levels similar to V exons. Thus, it is the genomic location, exons encoding for V regions, that matter for AID targeting. A wide spectrum of possible mechanisms has been proposed, including AID recruitment motifs distinct from that of the RGYW motif, enhancer activity, and ongoing ncRNA transcription. Now that Yeap et al. (2015) have resolved the role of RGYW motifs in AID targeting, the search begins to understand why and how the AID targeting machinery senses genomic location.

REFERENCES

Alt, F.W., Zhang, Y., Meng, F.L., Guo, C., and Schwer, B. (2013). Cell *152*, 417–429.

Chandra, V., Bortnick, A., and Murre, C. (2015). Trends Immunol. *36*, 527–535.

Di Noia, J.M., and Neuberger, M.S. (2007). Annu. Rev. Biochem. *76*, 1–22.

Hackney, J.A., Misaghi, S., Senger, K., Garris, C., Sun, Y., Lorenzo, M.N., and Zarrin, A.A. (2009). Adv. Immunol. *101*, 163–189.

Muramatsu, M., Nagaoka, H., Shinkura, R., Begum, N.A., and Honjo, T. (2007). Adv. Immunol. *94*, 1–36.

Nussenzweig, A., and Nussenzweig, M.C. (2010). Cell *141*, 27–38.

Odegard, V.H., and Schatz, D.G. (2006). Nat. Rev. Immunol. *6*, 573–583.

Yeap, L.-S., Hwang, J.K., Du, Z., Meyers, R.M., Meng, F.-L., Jakubauskait≐, A., Liu, M., Mani, V., Neuberg, D., Kepler, T.B., et al. (2015). Cell *163*, this issue, 1124–1137.

Zarrin, A.A., Alt, F.W., Chaudhuri, J., Stokes, N., Kaushal, D., Du Pasquier, L., and Tian, M. (2004). Nat. Immunol. *5*, 1275–1281.